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Autogenous vaccine therapy for condyloma acuminatum

A double-blind controlled study

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SUMMARY In an attempt to substantiate claims that autogenous vaccine is an effective form of immunotherapy for condyloma acuminatum, a double-blind cross-over study was carried out on 34 patients, in which autogenous wart vaccine was compared with a placebo vaccine identically prepared from each patient's own normal skin. It was found that the duration of disease was an important determinant of curability in patients with condylomata acuminata ($P<0.001$) and that when this factor was taken into account autogenous wart vaccine was not significantly more effective than the placebo ($P=0.43$).

Introduction

Condyloma acuminatum is a contagious, unsightly, and often persistent problem seen by a wide variety of medical and surgical practitioners. Caused by the human papilloma virus (HPV), this disease represents the only known virally induced tumour in man,[1,2] and cases of malignant transformation—although rare—have been reported.[3] While definite causality has not been proved in these cases, the presence of this disease may identify a host of greater susceptibility to certain forms of cancer.[4]

Evidence has recently been published which differentiates the HPV associated with common warts from that of the genital lesions on the basis of viral DNA homology studies.[5] Antigenically, however, the differences are not so distinct.[6,7] At present, confusion still exists about the roles played by the various components of the immune system in the resolution of human wart disease. This confusion is perhaps a reflection of the underlying complexity of the problem, and only in the last few years has the immunology of human wart disease captured the interest of researchers as a potential model for the study of human tumour immunology.

Historically, however, the first attempted application of immunology to the problem of human wart disease occurred over half a century ago. Biberstein, in 1925, theorised that the remission of warts was somehow linked to the host's immune system.[8] In an attempt to stimulate the immune system he injected his patients with a crude wart extract and attributed the resulting high cure rate to an evoked immune response. A dispute in the literature ensued when Cormia[9] could not reproduce Biberstein's results in 1934. This, and the publication of excellent results in the treatment of condylomata with podophyllin in 1944 by Culp and Kaplan,[10] caused Biberstein's technique to be largely forgotten.

Powell revived Biberstein's "vaccination" technique for treating condylomata acuminata in 1970 and reported cures in 23 out of 24 patients with lesions refractory to conventional measures.[11] Powell suggested that the wart virus was relatively "insulated" from the immune system in the superficial layers of the epithelium of the wart and that systemic injection of the wart extract provided more direct exposure of the antigen resulting in enhanced immunological destruction of the remaining wart tissue. Others have reported similar encouraging results with autogenous vaccine therapy in the past decade; however, none of these studies has included a control population of patients.[12-16]

Given the capricious nature of wart disease, it seemed desirable to carry out a study to evaluate the effectiveness of autogenous vaccine therapy for condyloma acuminatum in which the bias from spontaneous remission and placebo effect, or both, could be separated.

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Patients and methods

STUDY POPULATION

Thirty-four patients (23 male and 11 female) with condylomata acuminata in various locations (table I) were studied prospectively in a double-blind cross-over fashion. The duration of disease ranged from a few months to 35 years with a mean of 3·6 years. Twenty-one patients had had warts for less than a year, nine for 1-5 years, and four for more than five years. In 18 (53%) patients previous forms of conventional therapy, which included podophyllin, fulguration and simple excision, had failed; the remainder of the patients had not been treated previously (table II).

TABLE I *Distribution of condylomata acuminata by sex and location*

Patients	*Genital*	*Anal*	*Both*	*Total*
Male	8	7	8	23
Female	7	0	4	11

TABLE II *Previous methods of treatment for 18 patients with refractory lesions*

Treatment	*No of patients*
Podophyllin	17
Cryosurgery	5
Fulguration	4
Topical 5-fluorouracil	2
Simple excision	2
Combined therapies	8

Before enrolment in the study, patients were screened for gonorrhoea and syphilis. Three cases of gonorrhoea were detected and treated .

PREPARATION OF VACCINE

After informed consent had been obtained a sufficient quantity of wart tissue was excised (0·5-1·0 g) from each patient to produce an autogenous vaccine according to the method of Powell *et al.*[11] A specimen was submitted in each case for histological confirmation of the diagnosis. A similar quantity of normal skin was also excised from the non-dominant elbow region of each patient for the production of a placebo vaccine by an identical method, care being taken to prevent cross-contamination of specimens both at operation and in the laboratory.

Laboratory technique

Tissue was collected in sterile Hank's buffered saline solution containing 100 μg/ml gentamicin and transferred to the laboratory where it was either prepared immediately or stored frozen at −70°C for preparation the next day. Fresh or thawed tissue was minced with a sterile scalpel and placed in a centrifuge tube with sterile saline at a ratio of 9:1 (saline-to-tissue) (10% by weight). This mixture was then homogenised in a Ten-Broek tissue grinder and subsequently frozen and thawed four times in dry ice/alcohol and a 37°C water-bath. The resulting suspension was clarified by centrifugation at 5000 rev/min for 10 minutes. The supernatant was collected, inactivated by heating at 56°C for one hour, and recentrifuged at 10 000 rev/min for 10 minutes. The supernatant was collected as the vaccine, filtered through a 0·45 μ Millipore filter, and tested for sterility before administration. The final protein concentration of the vaccines varied considerably and ranged from 12 to 157 mg/ml with a mean of 57·3 mg/ml.

A limited number of vaccine preparations was centrifuged at 50 000 rev/min, the sediment stained with 2% phosphotungstic acid and examined by electron microscopy for the presence of viral-type particles. Several wart specimens were fixed, embedded in Epon 12, sectioned, and examined by electron microscopy for intranuclear virus-type particles (fig 1).

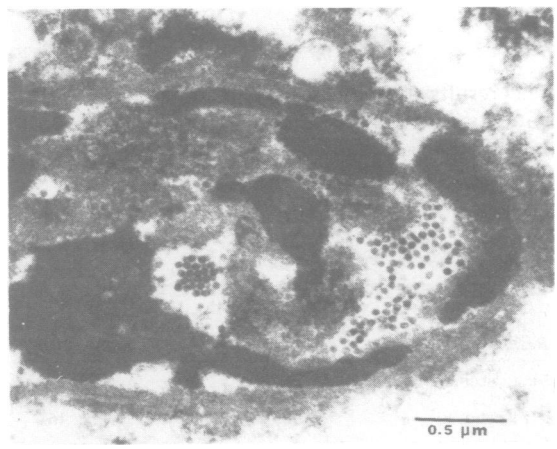

FIG 1 *Electron micrograph of intranuclear virus particles in a keratinocyte from a venereal wart.*

ALLOCATION OF THERAPY

A schematic diagram of the study plan is shown in fig 2. Patients were randomly selected by lottery to receive first either placebo or wart vaccine. The coded vaccines, identical in appearance, were administered as weekly subcutaneous injections of 0·5 ml over the deltoid region for six weeks.[16] All vaccines were stored frozen and thawed just before each administration. A positive response usually occurred before completion of the first series of

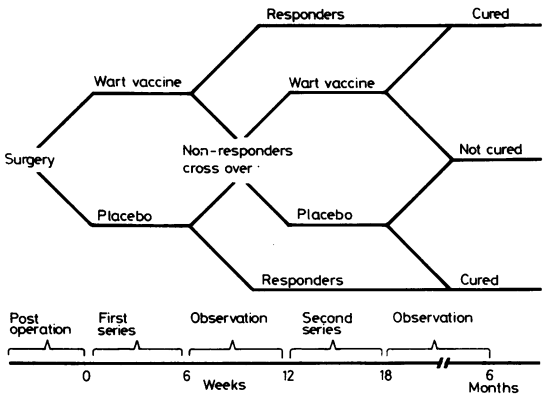

FIG 2 *Schematic representation of study plan*

injections. Those who did not respond, however, were observed for an additional six weeks before crossing over to the second series of vaccinations.

Patients were considered cured if there was no evidence of disease six months after treatment.

STATISTICAL ANALYSIS

The χ^2 test with Yates's correction was used.

Results

The results are summarised in table III. Eighteen patients were treated initially with autogenous wart vaccine and 10 (56%) were cured. Sixteen were initially treated with placebo vaccine and four (25%) were cured ($\chi_1^2 = 2 \cdot 12$; $P = 0 \cdot 14$).

TABLE III *Summary of clinical results*

	No of patients		
Results	*Cured (%)*	*Failed*	*Total*
First vaccine			
Wart	10 (56)	8	18
Placebo	4 (25)	12	16
Second vaccine			
Wart	2 (25)	10	12
Placebo	2 (17)	6	8

At cross-over the 12 patients who failed initially to respond to the placebo vaccine were treated with wart vaccine, and two (17%) were cured. Eight patients who failed initially to respond to wart vaccine were treated with placebo and two (25%) were cured. An equivalant P value cannot be directly calculated for the cross-over groups since the patients were no longer randomised because they were pre-selected by the outcome of the first series of vaccinations. It is apparent, however, that there was even less difference between the treatment results in the cross-over groups.

All patients responded in an all-or-none fashion; there were no partial regressions or incomplete cures.

The mean follow-up period is now 1·3 years with no reported recurrences.

No correlation was observed between the concentration of protein in the vaccines and the patients who were cured.

When the influence of duration of disease is taken into account, the data can be re-examined to compare the wart and placebo vaccines in only those patients with disease for one year or less (table IV). As expected the cure rates for all groups were higher regardless of the form of treatment, but, more importantly, there was much less difference between the two therapies than in the previous analysis, where duration of disease had not been considered ($\chi_1^2 = 0 \cdot 63$; $P = 0 \cdot 43$).

TABLE IV *Results in patients with disease for one year or less*

	No of patients		
Results	*Cured (%)*	*Failed*	*Total*
First vaccine			
Wart	10 (77)	3	13
Placebo	4 (50)	4	8
Second vaccine			
Placebo	2 (66)	1	3
Wart	2 (50)	2	4

No virus-type particles were observed in sediment from vaccines which had been negatively stained by phosphotungstic acid and examined by electron microscopy. A few wart specimens from patients with a disease duration of less than a year had intranuclear virus-type particles present.

Discussion

At first glance, a P value of 0·14, although not statistically significant, suggests that wart vaccine may have proved clinically superior to placebo had more subjects been added to the study population. On closer examination of the data, however, little difference was found between the two therapies in the cross-over groups. In addition, there is an unexpected disparity between the initial and cross-over groups treated with wart vaccine (56% against 17% cure rates, respectively), suggesting the possibility of an unanticipated bias which could have skewed the data.

When such a bias was looked for the important influence of the duration of disease was evident. In all patients with disease for one year or less the confirmed cure rate with vaccine or placebo was 86% (18/21) compared with zero (0/13) for those with the disease for more than a year ($\chi_1^2 = 25 \cdot 6$; $P < 0 \cdot 001$). Thus, this factor alone is capable of influencing the cure rates of different groups of patients treated by identical methods. It was subsequently discovered that the initial group treated with wart vaccine comprised 72% of patients with disease for one year or less compared with 33% for the cross-over group. Similarly, the overall cure rate in this study was only 56% (18/34) with the mean duration of disease being 3·6 years. This is in contrast to the 94% cure rate reported by Abcarian and Sharon[16] in a large series of patients treated by autogenous wart vaccine where the mean duration of disease was 0·5 years.

Is it possible that cure rates as high as 94% are attributable to either spontaneous remission or placebo effect? When one considers the results of the many studies performed in the 1920s and 1930s on patients with warts using various placebo therapies, this possibility does not seem unlikely. Allington[17] reviewed these works and reported rates of spontaneous remission and placebo cures ranging from 20-89%.

But this is not to say that the immune system does not play a role in the resolution of wart disease. On the contrary, most evidence available today, both serological and histological, indicates that it does. Still, it is not clear what specific factors are capable of influencing the immune system in patients with wart disease or, even less clearly, the mechanisms by which they do so. Autogenous wart vaccine once held promise as one of these specific factors; unfortunately, this theory has not been substantiated by a double-blind controlled study.

In summary, autogenous wart vaccine could not be shown to be more effective for condylomata acuminata than placebo vaccine prepared identically from normal skin. Conversely, it cannot be ruled out that some other antigen common to both normal skin and to condylomata is not responsible for such an action. This possibility would seem unlikely, however, in view of previous studies which have shown comparable cure rates in patients with warts treated solely by injections of saline[18] or distilled water.[19]

Our electron microscope examination of wart tissue showed relatively few cells containing intranuclear papilloma virus-type particles. The vaccines prepared for use in this investigation may thus have had low antigenicity. If it is possible to produce sufficient viral antigen by cell culture or DNA replication methods in the future, then vaccines with sufficient potency to provoke wart involution may result.

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